Citation:

Trichopoulou A, Psaltopoulou T, Orfanos P, Hsieh CC, Trichopoulos D. Low-carbohydrate-high-protein diet and long-term survival in a general population cohort. *Eur J Clin Nutr.* 2007 May; 61(5): 575-581.

PubMed ID: 17136037

Study Design:

Prospective Cohort Study

Class:

B - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To examine the mortality of individuals, healthy at enrollment, who participated in a population-based cohort study [the Greek component of the European Prospective Investigation in Cancer and Nutrition study (EPIC)] according to their carbohydrate (CHO) and protein intake.

Inclusion Criteria:

Inclusion criteria for the EPIC study are described elsewhere.

Exclusion Criteria:

- Missing information on one or more of the dietary, anthropometric or lifestyle variables
- Those subjects not actively being followed up (due to residence in remote areas of Greece)
- Participants who had coronary artery disease, diabetes mellitus, cancer or a combination of these diseases at enrollment
- Other EPIC Study exclusion criteria are described elsewhere.

Description of Study Protocol:

Recruitment

Subject recruitment for the EPIC study is described elsewhere. This study includes subjects recruited between 1993 and 1999 from all regions of Greece.

Design

Prospective cohort study in which follow-up was performed from 1993 to 2003 to evaluate the

effects of diet on mortality. Participants were distributed by increasing deciles according to protein intake or CHO intake, as well as by an additive score generated by increasing decile intake of protein and decreasing decile intake of CHOs.

Dietary Intake/Dietary Assessment Methodology

- Dietary data was collected using an interviewer-administered, food-frequency questionnaire (FFQ) including approximately 150 foods and beverages commonly consumed in Greece. The questionnaire was validated during the pilot phase of the EPIC study, and the interview focused on the year prior to enrollment
- Nutrient intakes were calculated by using a food-nutrient composition database appropriate for the Greek diet.

Statistical Analysis

- Distributions of study participants at enrollment by non-nutritional variables, as well as saturated and unsaturated lipids, CHOs, protein and total energy intake, were examined separately for men and women
- All study participants were classified by deciles of absolute and energy-adjusted protein and CHO intake. Energy adjustment was performed using the residuals method. For each participant, ascending decile of protein intake and descending decile of CHO intake were added to create an additive LC/HP using absolute and energy-adjusted CHO and protein amounts. (A subject with a LC/HP score of two is one with very high intake of CHOs and very low protein intake. A subjects with LC/HP score of 20 is one with a very high intake of protein and very low CHO intake.)
- A matrix of Spearman's correlation coefficients among principal groups of energy-generating nutrients, energy intake and the LC/HP score was generated
- Proportional hazards regression was used to assess the hazard of death in relation to CHO and protein intake. The following factors were controlled for gender, age at enrollment, years of schooling (i.e., socioeconomic status), smoking habits, BMI, physical activity, ethanol intake, total energy intake and saturated or unsaturated lipids
- In the Cox models, the assumption of proportionality was met, there was no collinearity among the variables used and no time-dependent covariates were used.

Data Collection Summary:

Timing of Measurements

- Participants were enrolled between 1993 and 1999, and followed through December 2003
- Dietary intake data was based on each subject's year prior to enrollment
- Lifestyle and sociodemographic variables were assessed at enrollment; it is unclear whether additional measures were taken following enrollment during the follow-up period.

Dependent Variables

Mortality was measured using date and cause of death determined from death certificates and other official sources, and classified based on the International Classification of Diseases, 10th revision.

Independent Variables

Dietary intake of CHO and protein were measured using a validated 150-item FFQ.

Control Variables

The following lifestyle and sociodemographic variables were used as controls. Information regarding these variables was collected with self-reported questionnaire.

- Gender
- Age at enrollment
- Years of schooling (used as a proxy for socioeconomic status)
- Smoking habits
- BMI
- Physical activity
- Ethanol intake
- Total energy intake
- Saturated or unsaturated lipid intake.

Description of Actual Data Sample:

Initial N

N=28,752 women and men

Age

20 to 86 years.

Attrition (final N) N=22,944

- 844 (3%) were excluded because information was missing for one or more of the dietary, anthropometrics or lifestyle variables
- 1,397 (5%) were not followed up due to residence in remote areas of Greece
- 3,387 (13%) were excluded because at enrollment they had coronary artery disease, diabetes mellitus, cancer or a combination of these diseases.

Subject Characteristics at Baseline

Variables		Men (N %)	Women (N %)
Age (years)	<45	3,249 (34.9)	4,247 (31.2)
	45 to 54	2,355 (25.3)	3,437 (25.2)
	55 to 64	1,888 920.3)	3,281 (24.1)
	≥65	1,820 (19.5)	2,667 (19.6)
Education (years)	<6	1,135 (2.2)	3,121 (22.9)
	6 to 11	3,712 (39.9)	5,608 (41.4)
	12	1,115 (12.0)	2,194 (16.1)
	≥13	3,350 (36.0)	2,709 (19.9)
BMI (kg/m^2)	<25	1,858 (20.0)	3,471 (25.5)
	25 to 30	4,905 (52.7)	5,073 (37.2)

	≥30	2,549 (27.4)	5,088 (37.3)
Physical activity (METh per day)	<30	1,183 (12.7)	881 (6.5)
	30±35	3,838 (41.2)	5,572 (40.9)
	35±40	2,339 (25.1)	5,402 (39.6)
	≥40	1,952 (21.0)	1,777 (13.0)
Ethanol intake (g per day)	<10	4,396 (47.2)	12,408 (91.0)
	10±30	3,077 (33.0)	1,095 (8.0)
	≥40	1,839 (19.8)	129 (1.0)
Smoking status	Never	2,331 (25.0)	9,742 (71.5)
	Past	2,922 931.4)	1,090 (8.0)
	Current	4,059 (43.6)	2,800 (20.5)
Protein (g per day)	<50	539 (5.8)	2,329 (17.1)
	50±70	2,174 (23.4)	5,294 (38.8)
	70±90	2,982 (32.0)	4,083 (30.0)
	≥90	3,617 (38.8)	1,926 (14.1)
CHOs (g per day)	<140	552 (5.9)	2,218 (16.3)
	140±190	2,178 (23.4)	5,060 (37.1)
	190±240	2,872 (30.8)	4,014 (29.5)
	≥240	3,710 (39.8)	2,340 (17.2)
Saturated lipids (g per day)	<20	1,063 (11.4)	3,106 (22.8)
	20±30	2,673 (28.7)	5,139 (37.7)
	30±40	2,804 (30.1)	3,427 (25.1)
	≥40	2,772 (29.8)	1,960 (14.4)
Unsaturated lipids (g per day)	<40	362 (3.9)	1,414 (10.4)
	40±60	2,134 (22.9)	5,122 (37.6)
	60±80	3,299 (35.4)	4,570 (33.5)
	≥80	3,517 (37.8)	2,526 (18.5)
Energy intake (kJ per day)	<6,000	428 (4.6)	2,396 (17.6)
	6,000 to 7,999	1,783 919.2)	4,908 (36.0)
	8,000 to 9,999	2,650 (28.5)	3,837 (28.2)
	≥10,000	4,451 (47.8)	2,491 (18.3)

Location

Greece.

Summary of Results:

Findings Related to Mortality

- Mortality with respect to non-nutritional variables was as expected; mortality was higher among men than women, declined with increasing years of schools, higher physical activity and alcohol intake and increased with smoking and total energy intake
- **Model 1:** Increasing protein intake was significantly associated with total mortality, and CHO intake was associated with non-significant (NS) reductions in mortality. Total energy intake was not controlled for.
- **Model 2:** The LC/HP score was positively associated with mortality, but NS (P=0.14). Total energy intake was not controlled for
- **Model 3:** Mortality was significantly associated with reduction of energy-adjusted CHO intake and non-significantly with increasing protein intake. Total energy intake was controlled for. Model does not specify the complementary changes that have to be introduced for the preservation of total energy intake when CHOs and **protein change**
- **Model 4:** Increasing LC/HP score was significantly associated with mortality (P=0.001). Most appropriate model: Total energy intake was controlled for; increasing LC/HP score was unrelated to total energy intake.

Increasing Intake	Model 1[a]	Model 2[a]	Model 3[a]	Model 4[a]
CHOs (per decile)	0.97 (0.92, 1.02)			
Energy-adjusted CHOs[b] (per decile)			0.94 (0.89, 0.99)	
Protein (per decile)	1.13 (1.03, 1.23)			
Energy-adjusted protein[b] (per decile)			1.02 (0.98, 1.07)	
LC/HP[c] absolute values (per two units)		1.08 (0.97, 1.20)		
LC/HP[c] energy-adjust components (per two units)				1.08 (1.03, 1.13)
[a] All models are controlled for sex, age, years of schooling, smoking, BMI, physical activity and ethanol intake. Presented as mortality ratio (95% CI).				
[b] Adjusted through the residuals method.				
[c] The LC/HP (low CHO, high protein) score. A high score implies higher protein, lower CHO intake.				

- An increase in the LC/HP score by two units was associated with an increase in mortality by 8%

- Compared to a referent group of with a score six or more, the mortality ratio was 1.20 for a score between seven and nine, 1.42 for a score between 10 and 12, 1.56 for a score between 13 and 15, and 1.71 for a score 16 or more
- The mortality rations by two-unit increase in the LC/HP score were: For 193 cardiovascular deaths, 1.09; for 175 cancer deaths 1.07; and for 87 deaths from other causes (19 respiratory, 13 digestive, 24 other pathological, 31 injuries), 1.11.

Author Conclusion:

Individuals with habitual diets low in CHOs and high in protein tend to have higher overall mortality compared to individuals with habitual diets high in CHOs and low in protein. This increase in mortality was not concentrated to particular causes, but was significant only with respect to cardiovascular deaths.

Reviewer Comments:

- *Dietary intake was only assessed once at enrollment using a FFQ based on the previous year*
- *The authors note that in this study population, consumption of CHO, even at the low extreme of the distribution, was still higher than amounts recommended by low-CHO diets, and few individuals consumed more than 20% of their energy from protein. However, it is unlikely that having more data at the extremes of low-CHO and high-protein intake distribution would reverse the trends seen in the data*
- *Generalizability to the United States may be limited.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	N/A
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	N/A
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	N/A
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	N/A

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes

1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	Yes
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes

4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	Yes
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes

7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes